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II. The Effects of Lead Acetate on the Hereditary Constitution

BY

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GENETIC STUDIES ON STENOSTOMUM INCAUDATUM

II. THE EFFECTS OF LEAD ACETATE ON THE HEREDITARY CONSTITUTION

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SEVENTY-ONE FIGURES

INTRODUCTION

In an earlier paper ('30) the author described the origin, nature, and fate of differences among individuals of a clone of *Stenostomum incaudatum*, living under presumably favorable conditions. The present contribution deals with some genetic consequences of making a specific unfavorable alteration in the environmental conditions under which the organisms live.

Only two earlier attempts to alter the hereditary characteristics of an asexual metazoan by altering its environmental conditions are known to me. Štolc ('03), working on the oligochaete *Aeolosoma hemprichii*, found that the number of segments that regenerated after some had been amputated was often abnormal, but that the progeny of the individuals possessing an abnormal number of segments always developed the normal number. He also found that an abnormal number of segments developed in individuals from very old culture medium; but again the progeny in fresh medium invariably developed the normal number of segments. Lashley ('15),

¹ This paper was prepared for publication while Fellow of the National Research Council. The author desires to record his indebtedness to Prof. H. S. Jennings and Dr. Ruth S. Lynch for much valuable assistance and advice during the course of this work.

working on Hydra, found that differences in the amount of food produced differences between different sets of parent and offspring, but that the effects disappeared when the offspring of different sets were given the same amount of food. He also found that buds of parents which had been injured by cuts were affected by the reduced vitality of the parent, but that the effects did not persist. These workers thus failed to alter hereditary characteristics by altering environmental conditions.

In the present work the environment was altered by the addition of dilute lead acetate to the fluid medium in which the organisms lived. A compound of lead was employed because it has been claimed that the effects of lead are hereditary and cumulative in man and other organisms. This latter property ascribed to lead makes it favorable for the study of whether cumulative environmental effects such as are known to occur in the Protozoa occur also in Metazoa.

In addition to the extensive literature on lead poisoning in man, some experimental studies on lower organisms have been reported. Flinn ('29) fed lead carbonate to guinea-pigs and claimed that this caused a declining birth rate in successive generations of treatment. In support of this claim the necessary detailed data on birth rate are not given, but the statement is made that the lines died out in the third generation. Male fowl and rabbits were poisoned with lead acetate by Cole and Bachhuber ('14), who concluded that the progeny, though not subjected to the poison, were of lower vitality. In spite of certain genetic precautions exercised by these investigators, their results lose weight in view of their imperfect knowledge of the genetic constitution of the animals studied, the small number of animals examined, the great variation among these, and some compensatory tendencies in the data themselves. Mann ('23) and Muller ('28) subjected *Drosophila* to lead acetate, as well as to other chemicals, but discovered no increase in mutability or tendency to be abnormal. Harrison and Garrett ('26) impregnated the food of certain Lepidoptera with lead nitrate and claimed thereby

to have induced melanism, which was inherited as a simple mendelian recessive. This work has attracted much attention and deserves careful consideration. Its significance has been made questionable by the work of Mansbridge ('27). By repeatedly selecting the darkest individuals in each generation to perpetuate the stock, he obtained melanic forms in three species of Lepidoptera. One of these was the species in which Harrison and Garrett had obtained their most convincing results; in it no melanic forms had been observed in nature. With doubt thus thrown upon the genetic purity of their experimental stocks, it becomes important to consider the possibility of obtaining melanics in the remarkable ratios (26:1, 29:1, etc.) in which they obtained them. Such ratios could have been obtained if the melanism depended upon multiple factors, or if there were a highly selective mortality among the eggs, or if there were great difficulty in recognizing melanics. Harrison ('28) admits the difficulty of recognizing melanics in one of his species; his mortality data allow ample possibility for a highly selective mortality to yield the ratios he obtained; and Federley ('23) found that melanism does depend on multiple factors in one species and suggested that this condition is probably widespread among Lepidoptera. Thus, neither the fact that no melanics of one species were ever found in nature nor the fact that the melanics appeared in peculiar ratios excludes the possibility that the stocks used by Harrison and Garrett contained latent genes for melanism. Stadler ('28) obtained results of much interest in an attempt to ascertain whether salts of heavy metals could increase the effectiveness of x-rays in producing mutations in barley. The rate of mutation when x-rays were applied in the presence of nitrates of barium, lead, or uranium was found to be more than twice as great as the rate of mutation when x-rays were applied in the absence of these salts; but the numbers examined were too small to yield conclusive results. Thus, no conclusive experimental result as to the effectiveness of lead salts in producing hereditary changes has yet been obtained.

MATERIAL AND METHODS

The animals used in the present work were members of the same clone used in the work reported in the first paper of this series ('30). The methods of preparing the daily culture fluid and of concentrating in it great quantities of the food organism *Colpidium striatum* were also described in that paper. This concentrated fluid was divided into two parts. The part to be used for controls was diluted with an equal quantity of boiled spring water; the part to be used for experimental animals was diluted with an equal quantity of 0.005 per cent lead acetate from a stock solution. One drop of the appropriate fluid was a day's food for each specimen. Different sets of pipettes, depression slides, Petri dishes, and so on, were used for the two different treatments.

The lead acetate used in this work had the formula $\text{Pb}(\text{CH}_3\text{COO})_2 \cdot 3\text{H}_2\text{O}$. Three stock bottles have been used: the first, used in all work at the Zoölogical Laboratory of the Johns Hopkins University, included 0.002 per cent iron, 0.002 per cent chlorine, 0.002 per cent calcium oxide, a trace of sodium, and no copper; the second, used at the Marine Biological Laboratory, Woods Hole, Massachusetts, during the summer of 1926, included 0.003 per cent iron, 0.001 per cent chlorine, a trace of sodium, and no copper; the third, used at the same laboratory during the summer of 1927, included a trace of potassium, no chlorides, no iron, and no calcium. The experimental results obtained with these three stock bottles have been in complete agreement; therefore, no mention of the particular solution used in each experiment will be made.

As already mentioned, the standard concentration used was 0.005 per cent lead acetate, diluted half and half with culture fluid. In the early exploratory experiments, however, solutions of diverse concentrations were tried. Solutions stronger than 0.3 per cent were found to kill almost instantly. In solutions of 0.05 per cent lead acetate the worms lived, on the average, an hour and a half. However, solutions stronger than 0.005 per cent were lethal within twenty-four hours to

the food organism, *Colpidium striatum*. Therefore an effective concentration of 0.0025 per cent was used exclusively in all experiments here reported. Since new colpidia in new fluid are supplied to the worms daily, this concentration does not alter the quantity of food supplied.

To this standard concentration of lead acetate, the experimental animals were always subjected continuously during the period of treatment. Intermittent subjection to lead acetate was not attempted, because, if the lead affected differently individuals in diverse developmental stages (as seemed likely), it would have been very difficult, practically, to have obtained uniformity in this respect among the individuals of the groups compared, at the time of each successive treatment.

EFFECTS OF LEAD ACETATE ON INDIVIDUALS CONTINUOUSLY
SUBJECTED TO IT

*1. Effects of lead acetate on individuals subjected to it
throughout life*

In order to ascertain the effects of lead acetate on individuals subjected to it throughout life, an exploratory experiment was performed on two groups of twelve individuals each. One group was cultured in fluid containing lead acetate; the other group was cultured in fluid containing no lead acetate. At the beginning of the experiment, all individuals were less than twenty-four hours old and were the first offspring of two-day-old parents. (The terms 'individual,' 'parent,' and 'offspring' are used here with the meanings given in the first paper of this series. All successive anterior products of division of a line together constitute a single individual; the posterior product of each division is the offspring of the anterior, or parent.) The values for the mean of length of life, productivity (total number of offspring produced), and rate of reproduction of these two groups and the differences between these means are given in table 1. Statistical methods are obviously unable to establish the existence of small differences between groups composed of

such small numbers of individuals. Therefore the fact that the difference in mean rate of reproduction between the two groups is four times its probable error does no more than suggest that lead acetate may have produced a slight decrease in rate of reproduction. However, it is clear that whatever differences may have existed were small. Furthermore, the structural developments of the groups differed little. Indeed, allowing for individual variation in this respect, it cannot be said that the lead treatment made any alteration in structural development. Thus, the chief result of this experiment is the discovery that subjection to 0.0025

TABLE 1

Vital statistics of two groups of twelve individuals each, one subjected throughout life to 0.0025 per cent lead acetate and the other not subjected to lead acetate

	MEAN LENGTH OF LIFE (IN DAYS)	MEAN PRODUCTIVITY (NUMBER OF OFFSPRING)	MEAN RATE OF REPRODUCTION (NUMBER OF OFF- SPRING PER DAY)
Individuals of group subjected to 0.0025 per cent lead acetate throughout life	68.1 $\pm$ 2.3	99.7 $\pm$ 3.1	1.46 $\pm$ 0.02
Individuals of group not subjected to lead acetate	66.7 $\pm$ 1.9	105.6 $\pm$ 3.2	1.58 $\pm$ 0.02
Value for controls, minus value for lead-treated animals	-1.4 $\pm$ 3.0	5.9 $\pm$ 4.5	0.12 $\pm$ 0.03

per cent lead acetate continuously throughout life produced no great effect on individuals, although they were exposed to the lead for an average of nearly seventy days.

2. Effects of lead acetate on lines of successive first offspring

By subjecting lines of successive first offspring (lines of successive posterior products of division) to lead acetate, will later generations show a greater effect than earlier ones? In order to answer this question the rates of reproduction of successive first offspring subjected to lead acetate were studied in three experiments performed at different times. The individuals of successive generations were not kept in lead acetate throughout the whole duration of their lives; each individual was retained only until it had produced its

first offspring. Since this requires only about a day for normal individuals, it was possible in this way to follow a much greater number of generations in a given time than by observing the individuals of each generation during a large portion of their lives.

TABLE 2

Amount of treatment required to produce abnormality in 138 lines of successive first offspring

NUMBER OF GENERATIONS OF TREATMENT REQUIRED TO PRODUCE ABNORMALITY	NUMBER OF LINES ABNORMAL	DAYS OF TREATMENT REQUIRED TO PRODUCE ABNORMALITY	NUMBER OF LINES ABNORMAL
2	4	5	1
3	67	6	5
4	32	7	14
5	15	8	14
6	11	9	19
7	3	10	23
8	2	11	15
9	4	12	4
Total,	138	13	1
Mean, 4.0 ± 0.1		14	10
		15	7
		16	9
		17	4
		18	1
		19	3
		20	3
		21	1
		22	1
		23	1
		28	1
		29	1
		Total,	138
		Mean, 11.5 ± 0.4	

In these experiments other effects more striking than those on rate of reproduction were discovered. These will be described first. It was found that every one of the 247 lines observed in these three experiments became structurally abnormal after less than ten generations of subjection to the lead acetate. Among forty control lines not subjected to lead acetate, not a single one showed any structural abnormality.

All of the controls were observed for at least ten generations, many for twenty generations, and some much longer. In table 2 the number of generations and the time that the different lines had been subjected to lead acetate before structural abnormality appeared are given. This table includes only the data of the second experiment, but this is thoroughly typical of all three experiments. The mean number of generations of treatment required to produce abnormality was 4.0 ± 0.1 , and the mean time corresponding to this was 11.5 ± 0.4 days. The required treatment varied from two to nine generations and from five to twenty-nine days. The modes were three generations and ten days. After abnormality had been produced in the lines, they soon ceased to reproduce and died unless removed from the lead acetate.

The striking difference between these lines of successive first offspring (successive posterior products of division) and the reaction of single individuals (successive anterior products of division), described above on page 413, to 0.0025 per cent lead acetate strongly confirms the results reported in the first paper of this series: that there exist many differences between anterior and posterior products of division. Indeed, chronologically, the difference between these two types of lines in their reaction to lead acetate was the original observation that led to the work reported in the earlier paper. Together, the two papers show that in both 'normal' and abnormal environments anterior and posterior products of division are not equivalent.

The above-described production of abnormality and death in lines of successive first offspring subjected to lead acetate was obvious. The effect of the treatment on the rate of reproduction is less obvious, but, as will appear, no less real. The only possible measure of rate of reproduction was the mean time that was required for the individuals of each generation to produce their first offspring, because they were retained under observation only until this occurred. The times were noted for each generation until reproduction ceased. These data are given in table 3. In the first experi-

ment, during the five generations in which reproduction occurred, there was no evidence of a cumulative increase in the mean time for the individuals of successive generations to produce their first offspring. In the second experiment the mean increased steadily during the first three generations, but no regular change occurred during the remaining six generations. In the third experiment the mean time increased steadily from generation to generation, with the exception of the sixth and eighth generations. The means for

TABLE 3

Mean times (in days) for the individuals of successive generations to produce their first offspring

GENERATION	MEAN TIME (IN DAYS) FOR THE PRODUCTION OF THE FIRST OFFSPRING			
	Experiment 1	Experiment 2	Experiment 3	Total for all three experiments
1	1.8	2.6	1.5	2.1
2	1.3	3.7	1.7	2.8
3	1.2	4.5	2.5	3.5
4	1.4	3.8	2.9	3.3
5	2.0	3.9	3.0	3.5
6	..	3.4	2.9	3.2
7	..	3.8	4.0	3.9
8	..	3.0	3.5	3.1
9	..	3.0	..	3.0
Number of individuals	12	137	98	247

all 247 lines of these three experiments show a steady increase during the first three generations, but no regular change during the remaining generations.

However, the fact that abnormality was produced in some lines after only two generations of treatment and in other lines not until as much as nine generations of treatment shows that the lead acetate was acting more slowly on some lines than on others. It seemed likely, therefore, that the rate of reproduction might also have been affected differently in different lines, and that perhaps these differences between lines obscured one another when the average of the whole group

was examined. For this reason, the lines were arranged in classes on the basis of the number of generations of treatment before reproduction stopped. Class 1 includes only those lines in which reproduction stopped after three generations of subjection to the lead acetate; class 2, those in which it stopped after four generations; class 3, five generations, and so on. The mean times in which the individuals of successive generations produced their first offspring, when the lines are classified in this way, are presented in table 4. In the first four classes, containing 220 of the 247 lines, the

TABLE 4
Mean times for the individuals of successive generations to produce their first offspring

GENERATIONS	MEAN TIME (IN DAYS) FOR THE PRODUCTION OF THE FIRST OFFSPRING							
	Class 1	Class 2	Class 3	Class 4	Class 5	Class 6	Class 7	Class 8
1	2.5	2.2	1.8	2.0	1.7	2.2	2.0	2.0
2	4.8	2.7	2.1	2.3	1.7	2.8	2.7	2.5
3	..	4.1	2.9	3.0	2.6	2.8	2.7	2.0
4	..	..	4.0	2.6	2.2	3.0	3.3	2.5
5	..	..	..	4.0	2.4	3.6	2.6	3.5
6	..	..	..	..	3.6	3.0	2.7	3.0
7	..	..	..	..	..	4.0	3.7	4.0
8	..	..	..	..	..	..	3.0	3.5
9	..	..	..	..	..	..	..	3.0
Number of individuals	35	93	56	36	13	5	7	2

mean time steadily increased in successive generations, with the single exception of the fourth generation of class 4. In class 5 the third generation and in class 6 the sixth generation are exceptions to the steady increase in the means. In classes 7 and 8 there are numerous irregularities; only a general trend toward increasing means is apparent. These irregularities in the data may be partly due to the fact that observations were made only once a day. Thus, if division occurred in any line soon after the observation for that day had been made, the record for this line would be one day too great for the earlier generation and one day too short for the next

succeeding generation. In classes composed of small numbers of individuals, there is less likelihood that discrepancies of this sort will be compensated by equal discrepancies in the opposite direction (division in some lines just before the observation for the day is made). Thus, in classes 5, 6, 7, and 8 the mean of any particular generation could be greatly affected by this sort of thing. Moreover, the fact that effects were produced more gradually in these classes would also tend to make it more difficult to detect the increased effect

TABLE 5

Mean times for the individuals of successive groups of generations to produce their first offspring

GENERATION	MEAN TIME (IN DAYS) FOR THE PRODUCTION OF FIRST OFFSPRING			
	Class 5	Class 6	Class 7	Class 8
1	1.7	2.5	2.5	2.2
2				
3	2.4	2.9	2.9	3.0
4				
5	3.0	3.3	3.4	3.5
6				
7		4.0		
8				
9				
Number of individuals	13	5	7	2

on each succeeding generation. To overcome this difficulty, the mean times per generation were averaged in these classes for two or three generations together. These averages are given in table 5. They show clearly the steady increase in mean time of successive groups of generations. When the obscuring factors are disentangled in this way, the cumulative increase in time to produce the first offspring in successive generations becomes apparent.

As controls, forty lines not subjected to lead acetate were observed. Some of these were carried at the same time, others at different times from the lead-treated lines. In this

mixed set (and the same is true of the records of each group taken separately) the mean time to produce the first offspring in successive generations is practically constant (table 6). From minimum to maximum it varies only from 1.1 to 1.3 days. Some lines were followed for many more generations with the same result. The difference between the controls and the various classes of lead-treated lines in the mean time required to produce the first offspring is greater and greater in successive generations (table 6).

TABLE 6
Difference between controls and lead-treated animals in the mean time to produce first offspring in successive generations

GENERATIONS	MEAN TIME (IN DAYS) FOR CONTROLS TO PRODUCE FIRST OFFSPRING	EXCESS OF MEAN TIME (IN DAYS) REQUIRED BY LEAD-TREATED ANIMALS ABOVE THAT REQUIRED BY CONTROLS TO PRODUCE THE FIRST OFFSPRING IN SUCCESSIVE GENERATIONS							
		Class 1	Class 2	Class 3	Class 4	Class 5	Class 6	Class 7	Class 8
1	1.2	1.3	1.0	0.6	0.8	0.4	1.2		
2	1.3	3.5	1.4	0.8	1.0			1.3	1.0
3	1.2	..	2.9	1.7	1.8	1.2	1.7		
4	1.2	..	..	2.8	1.4				
5	1.1	..	..	..	2.9	1.9	2.2	1.8	1.9
6	1.1	..	..	..	..				
7	1.2	..	..	..	..	..	2.8		
8	1.2	..	..	..	..	..	..	2.2	
9	1.1	..	..	..	..	..	..	..	2.3
Number of individuals	40	35	93	56	36	13	5	7	2

Cumulative environmental effects such as these have been discovered in many other organisms (chiefly Protozoa); this condition has been interpreted by some investigators as evidence of the inheritance of environmental effects. With certain difficulties of this interpretation in mind, the author undertook a general analysis of this subject. As a result, he became convinced that such cumulative action can be explained at least as plausibly on the basis of non-inheritance as on the basis of inheritance. The details of this analysis will be communicated in a later paper. Meanwhile, inter-

pretation of the cumulative effects here reported is withheld until, by further experimentation, the relation that exists between the amount of the treatment with lead acetate and the duration of the effects when the individuals bearing them are removed to lead-free, control culture fluid is discovered.

3. The relation of the results obtained here to the results of other investigations on other animals

In Protozoa many workers have observed that the effects of certain environmental conditions become greater and greater as more and more generations are subjected to them. A summary of this work is given by Jennings ('29). In the asexual Metazoa no attempts—except the present one on *Stenostomum*—have been made to discover whether such conditions exist or not. In the parthenogenetic Metazoa positive results were obtained by Finesinger ('26) and Lynch and Smith (unpublished), working on Rotifera. In bisexual Metazoa, although numerous claims for the cumulative effects of environmental conditions acting on a succession of generations have been made, none of these has withstood the criticisms brought against it. Moreover, recent extensive studies made by Hanson and Heys ('27) on the effects of alcohol fumes and by Vicari ('29) on the effects of training have yielded only negative results. Consequently, in these organisms such cumulative action is generally held not to occur. It thus appears that, in this respect, *Stenostomum* resembles the Protozoa and Rotifera and differs from bisexual Metazoa.

THE FATE OF CERTAIN STRUCTURAL AND PHYSIOLOGICAL EFFECTS
OF LEAD ACETATE, AFTER INDIVIDUALS BEARING THEM HAVE
BEEN REMOVED FROM THE TOXIC ENVIRONMENT

1. The types of abnormalities produced by subjection to lead acetate for a number of generations

What was the fate, after permanent removal from culture fluid containing lead acetate, of the abnormalities produced by cultivating a number of generations in fluid containing lead acetate? Individuals were considered abnormal if they

possessed structural peculiarities different from those found among controls, or if they possessed the same peculiarities at different stages of the life history. Nearly all the abnormals were obtained by subjecting to lead acetate a series of first offspring of successive generations; hence, at the time they were discovered, these abnormals were all very young. It is important to keep this in mind, because the abnormals sometimes were, superficially at least, like normal individuals in advanced stages of decline. The abnormalities were, in general, of these types:

1. Abnormalities in reproduction: *a*) Cessation of elongation and reproduction. *b*) Dedifferentiation of division zones (fig. 1). *c*) Persistent attachment of zooids to the parental chain (fig. 2). *d*) Irregularity in the relative sizes of the zooids (fig. 3).

2. Abnormalities in growth: *a*) Outgrowths from the trunk; these contain all the usual trunk tissues (fig. 4). *b*) Surface bulges on the trunk; these appear to contain only epidermis (fig. 5). Abnormalities of types *a* and *b* may be comparable to the 'tumor-formation' observed in *S. leucops* and *S. unicolor* by Hartmann ('22). *c*) Corrugation of the surface of a limited region of the trunk (fig. 6). *d*) Thickening and darkening of a limited region of the trunk (fig. 7).

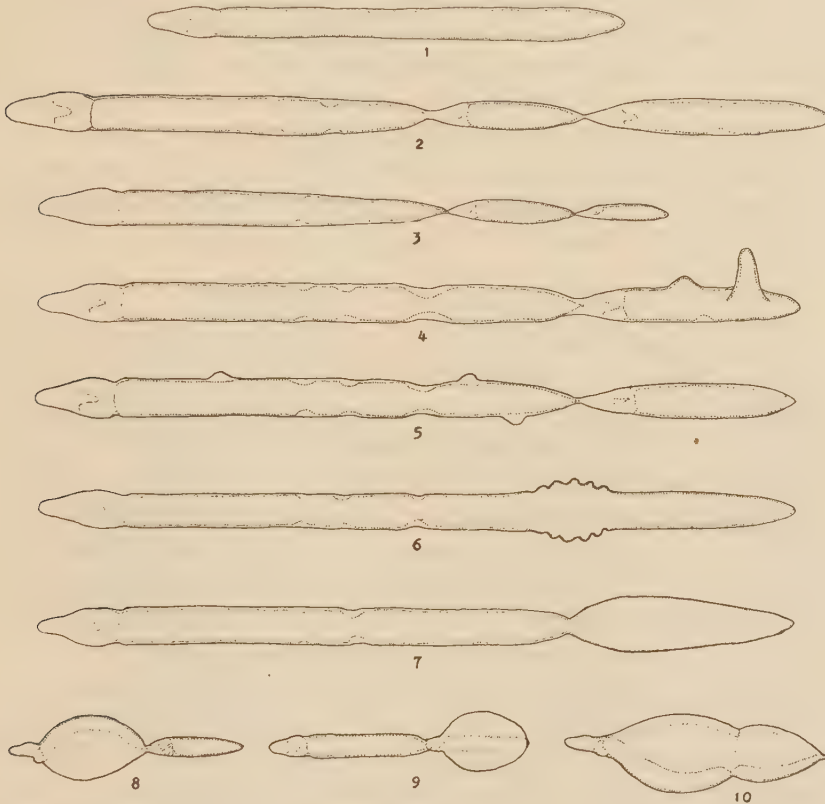
3. Abnormality in the size of the pseudocoel: *a*) Swelling of the pseudocoel in either the parental or zooidal part of the chain (figs. 8 and 9). *b*) Swelling of the whole pseudocoel (fig. 10).

These types of abnormalities are not mutually exclusive; some individuals possess first one type of abnormality, then another. For example, individuals of the types described in 1a, 1c, or 1d may later be of the types described in 3a or 3b above.

2. Mortality among the abnormals produced by the action of lead acetate

If lines of successive first offspring in which abnormalities had arisen remained in lead acetate, they invariably died out.

Even when they were removed to lead-free control culture medium, mortality was high. Of 138 individuals removed from lead acetate (ninety within one day, thirty-five within seven days, thirteen within twenty days of the first appearance of abnormality), eighty-nine, or 64.5 per cent, died.



Figs. 1 to 10 Types of abnormalities produced by subjecting lines of successive first offspring to 0.0025 per cent lead acetate. These diverse types are classified and characterized on page 422

3. Fate of the surviving abnormalities

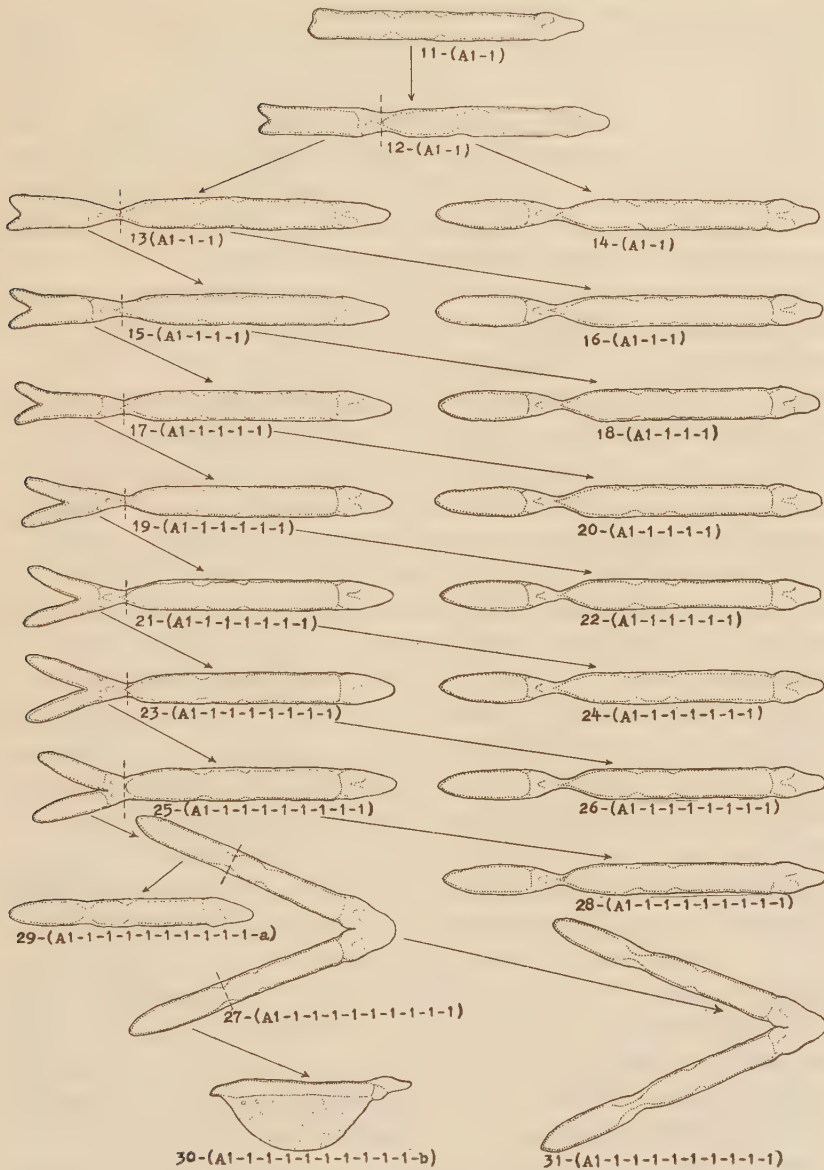
Of the forty-nine surviving abnormalities, none remained abnormal. Return to the normal condition came about either by the gradual disappearance of the abnormality or by the transmission of the abnormal parts to offspring. The latter

invariably occurred if the abnormal parts were restricted to the posterior end of the body and if reproduction continued. For example, abnormalities like 1a and 1b, and some like 2b, 3a, and 3b, gradually disappeared on removal from lead acetate; others, like 1c and 1d, disappeared as soon as the abnormal offspring separated from their parents; others, like 2a, and some like 2b and 3a, were simply transmitted to offspring as a consequence of their position near the posterior end of the body. None of the surviving individuals permanently retained its abnormality, because none of these abnormalities affected the pre-intestinal region of the body. The permanence of abnormal structural characters depends largely on their location anterior to the intestine.

4. *The progeny of the abnormals*

A. The origin and extinction of non-persistent abnormal stocks. Among the progeny of the forty-nine surviving abnormals, there were to be found both normal and abnormal individuals. No abnormal offspring were produced by the original abnormals after these had become normal. Even before becoming normal, some of the original abnormals, like types 1a, 1b, 1c, or 1d, never produced any abnormal offspring. Most of the original abnormals, however, produced both normals and abnormals. Abnormals arose and died out in the two ways illustrated by the following two typical histories.

The first history illustrates how a single structure was sometimes transmitted successively to a single individual in each of a series of generations. This particular series of generations began with the individual A, that entered lead acetate on the 6th of July, 1927. The first individual of the third treated generation, A1-1 (descended from A through A1, its first offspring), was produced on July 12th. This individual failed to grow during the first four days of its life and developed a slightly enlarged pseudocoel on the fifth day. It was then removed permanently from lead acetate into lead-free culture medium. Next day, the pseudocoel had regained its normal size, but a slight projection was present



Figs. 11 to 31 Origin and fate of bifurcation of posterior part of trunk. Broken lines indicate division planes. Arrows point from earlier to later stages of the same individual. After the number of each figure, the name of the individual represented is given in parentheses. The detailed history of this line is given on page 424.

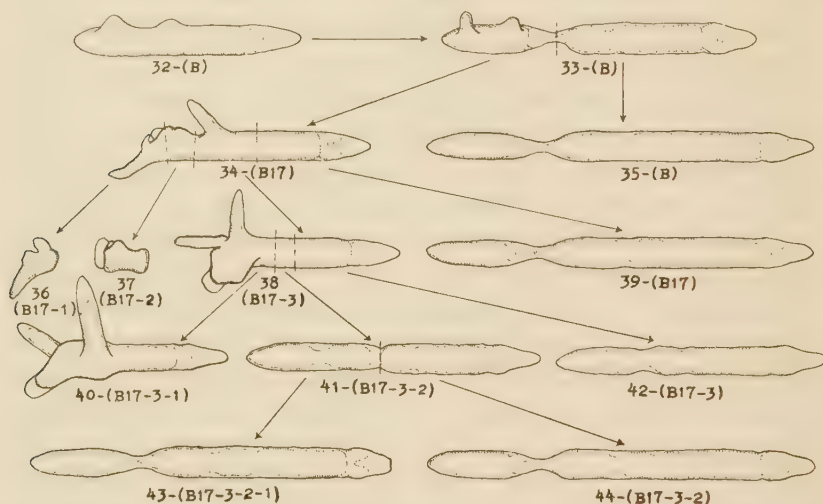
near the posterior end of the body (fig. 11). On the following day this projection had grown; the posterior end of the body now resembled a two-pronged fork (fig. 12). Division zones arose anterior to the prongs, so that the first offspring A1-1-1, which separated from its parent (A1-1) on July 19th, contained the fork at its posterior end (fig. 13). The parent, A1-1, thus became and remained normal (fig. 14). As shown in figures 15, 17, 19, 21, 23, 25, 27, the fork became the terminal portion of successive first offspring for seven more generations, repeatedly leaving the parents normal (figs. 16, 18, 20, 22, 24, 26, 28). Meanwhile, in successive generations the prongs of the fork elongated and the region between the head and the point of convergence of the prongs was reduced (because more uncleft trunk is cut off by fission than is reformed by subsequent growth of the new offspring). Eventually, this point of convergence of the prongs lay anterior to the intestines (fig. 25); therefore, since division zones do not arise in pre-intestinal tissues, all of the uncleft portion and part of the two prongs remained permanently in the same individual, A1-1-1-1-1-1-1-1-1-1 (figs. 27 and 31). Each prong of this forked individual produced, by division, single unforked offspring; half of all those produced were of normal form, like A1-1-1-1-1-1-1-1-1-1-a (fig. 29), and lived; the other half developed enlarged pseudocoels, like A1-1-1-1-1-1-1-1-1-1-b (fig. 30), and died. Abnormality of this line ended with the death of the one permanently 'forked' individual. It thus appears that an abnormal structure, 'forked,' may be transmitted successively to nine individuals of nine generations (figs. 12, 13, 15, 17, 19, 21, 23, 25, 27). It is to be noted that at any given time only a single individual possessed the abnormal character 'forked.' This fact is the distinguishing characteristic of this type of transmission. It is obviously a consequence of normal elongation accompanied by the normal formation of division zones anterior to the abnormal region; it is indistinguishable from the process of transmission observed among old individuals under 'normal' conditions described in the first paper of this series.

The second history illustrates the transmission of parts of structures to several individuals in each of one or more generations. The individual B had been in lead acetate (of unknown concentration, but probably greater than 0.0025 per cent) for sixteen days when it was permanently removed to lead-free culture fluid on August 10th. By this time, it had developed two lateral outgrowths (fig. 32), which were considerably enlarged (fig. 33) by the next day. On August 12th, the whole abnormal region became part of the seventeenth zooid, B17, leaving B normal (fig. 35). One of the outgrowths had developed into a lateral zooid of reversed polarity; the other had become very irregular in form (fig. 34). These abnormalities of B17 were completely transmitted to offspring, so that after three divisions B17 was normal (fig. 39). Its first two offspring, B17-1 (fig. 36) and B17-2 (fig. 37), were both irregular in form, but in quite different ways. Both soon died without descendants. The third offspring, B17-3 (fig. 38), received all the remaining abnormal structure of its parent; these consisted of several of the irregular outgrowths and of the lateral zooid with reversed polarity. After dividing its abnormalities between its first two offspring, B17-3-1 (fig. 40) and B17-3-2 (fig. 41), it became normal (fig. 42). Its first offspring received the irregular outgrowths and lateral zooid and soon died without descendants; the second possessed an unusually long posterior zooid. This developed into its first offspring, B17-3-2-1 (fig. 43), and possessed an abnormally blunt, or truncate, head. All other progeny of the line were normal.

The history of this line illustrates the following points:

- 1) More than one individual at a time may be abnormal: for example, on August 13th, B17 and B17-1 were abnormal.
- 2) More than one individual in one generation may be abnormal: for example, in the third generation, B17-1, B17-2, and B17-3; and in the fourth generation, B17-3-1 and B17-3-2.
- 3) More than one type of abnormality may be developed by the descendants: for example, simple outgrowths from the trunk, by B; lateral zooid with reversed polarity, by B17,

B17-3, and B17-3-1; irregular form, by B17, B17-1, and B17-2; unusually long zooid, by B17-3-2; truncate head, by B17-3-2-1. 4) When transmission of abnormal structure to offspring occurs, it need not be completed in one step; that is, part may be transmitted to one offspring, part to another: for example, a part of the abnormal structures of B17 was transmitted to B17-1, part to B17-2, and part to B17-3. 5) Sometimes transmission of abnormal structures to offspring does



Figs. 32 to 44 Origin and fate of abnormality in the line descended from the individual B. Broken lines indicate division planes. Arrows point from earlier to later stages of the same individual. Along with the number of each figure, the name of the individual represented is given in parentheses. The detailed history of this line is given on page 427.

not occur at all. This is the situation when the abnormal individuals die without producing any offspring: as was the case with B17-1, B17-2, and B17-3-1; or when the abnormality is restricted to the pre-intestinal part of the body: as was the case with B17-3-2-1. In the latter case the abnormal individual produced many offspring, all normal.

The diverse sorts of abnormal individuals that arose in this line developed as a consequence of the division of the abnormal structures of a single individual among several offspring;

further, the abnormal structures continued to grow and differentiate during this process of redistribution. The same type of transmission occurred among old declining individuals under 'normal' conditions (see first paper of this series). In the case of the line descended from the individual B, all of the abnormals were derived from material originally at the posterior end of the first abnormal individual, B; material that continued to grow and differentiate throughout this whole process involving five generations. That no fundamental change in the constitution was produced is shown by the fact that all individuals derived from other material than that located in the posterior part of B were perfectly normal.

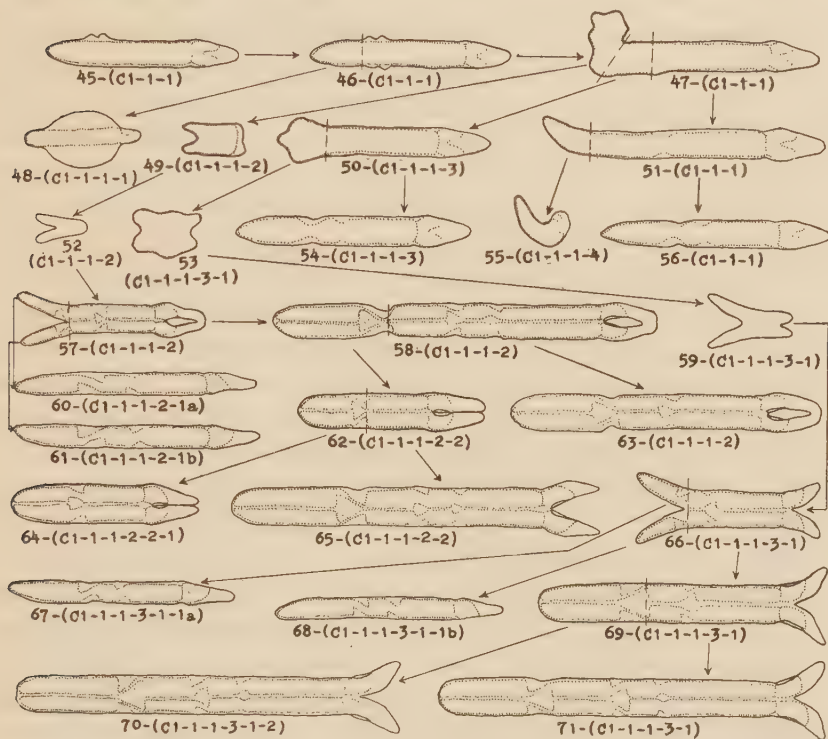
Up to this point we have focused attention on the ways in which non-persistent abnormal stocks arose. Why was it not possible to establish permanently abnormal lines by selection of the abnormals? A review of the two histories just described will show that failure to establish permanently abnormal lines was due to several conditions: 1) The abnormals either died without offspring, or gradually became normal by regulation or transmission, or remained permanently abnormal. 2) Those that gradually became normal then produced only normal offspring. 3) In those that remained permanently abnormal, the postpharyngeal region became normal, and then these individuals produced only normal offspring. 4) The small number of abnormal progeny thus produced had fates like those mentioned above in 1. In later generations, however, more and more of the abnormal progeny either died without offspring or produced very few abnormal offspring, so that eventually all abnormals died out. 5) The normal offspring that arose from the abnormals produced only normal progeny. Thus, after a short time, only normals were left. In this change of abnormal populations into normal ones, the main feature is not the regulation of the abnormalities, but extinction of the abnormals after new normal lines have been established. The same result, from the same sort of processes, occurred among the unusual progeny of old declining parents, as described in the first paper of this series.

B. The origin, fate, and nature of persistent abnormal stocks. In addition to the transient abnormalities described in the preceding section, two permanently abnormal stocks arose. The detailed histories of these two stocks follow.

The line derived from the individual C entered lead acetate on July 6, 1927. The individual C1-1-1 of the fourth generation in lead acetate was produced on July 17th. This individual developed slight epidermal bulges on its trunk (fig. 45) on July 20th, and was then permanently removed to lead-free control culture fluid. From July 18th to July 23rd no elongation took place, but the tissues in one part of the body became bunched together (fig. 46). The part of the body posterior to this bunching separated as the first offspring, C1-1-1-1 (fig. 48). This had an enlarged pseudocoel and died in two days. The posterior part of the parent now looked like an L (fig. 47). Part of this L separated as the second offspring, C1-1-1-2 (fig. 49), which was an irregular, almost structureless mass; the remainder of the L became the posterior end of the third offspring, C1-1-1-3 (fig. 50). After this, the parent, C1-1-1, had an enlarged pseudocoel for a few days and then developed an abnormal posterior end (fig. 51). This abnormal region separated as the fourth offspring, C1-1-1-4 (fig. 55), which soon died. Thereby, C1-1-1 became normal (fig. 56), remained so, and produced only normal progeny.

One new race developed from the irregular, structureless individual, C1-1-1-2 (fig. 49), and another new race developed from a similar irregular, structureless individual, C1-1-1-3-1 (fig. 53), which had separated from C1-1-1-3 as its first offspring. These two individuals went through a similar development. Both became smaller and paler; both were marked by terminal prominences: two at one end of C1-1-1-2 (fig. 52) and two at each end of C1-1-1-3-1 (fig. 59). Eventually, after a week of little or no change, both individuals began to grow. The two prominences of C1-1-1-2 developed into the two prongs of a fork at the posterior end (fig. 57); in each prong there was a branch of the intestine. Through-

out the body, there was a complete double set of organs: two intestines, two pharynges, two sets of brain ganglia, and so on. These two sets were contiguous except at the posterior end, where the prongs of the fork diverged, and at the region



Figs. 45 to 71 Origin of two different races of individuals with double sets of structures. Broken lines indicate division planes. Arrows point from earlier to later stages of the same individual. After the number of each figure, the name of the individual represented is given in parentheses. The detailed history of this line is given on page 430.

of the mouths, where there was a short cleft between the two heads. The parts were so disposed that the surfaces which would have been ventral in individuals with a single set of structures faced or touched each other in this double individual. Each prong of the posterior 'fork' separated as a single individual with no double structures (figs. 60 and 61),

leaving the two sets of structures of C1-1-1-2 completely contiguous except for the cleft near the mouths (fig. 58). All later progeny of C1-1-1-2 were exactly alike, as shown in the diagrams of C1-1-1-2-2 (fig. 62) and C1-1-1-2-2-1 (fig. 64). They differed from the parent, C1-1-1-2 (fig. 63), only in the way the heads were joined: when young, the heads were closely conjoined (figs. 62, 64), but when mature, there was complete separation of the independently moving heads from the anterior end to the base of the mouth (fig. 65). During the period of observation (August 8th to August 26th), these were cultivated for ten generations, during which 188 individuals of this race were produced.

The two anterior prominences of C1-1-1-3-1 turned out to be two heads, and the two posterior prominences, the two prongs of a 'fork' (fig. 66). Again a complete double set of organs was present, but the arrangement was such that the surfaces which would have been dorsal in individuals with single sets of organs faced or touched each other in this double individual. The prongs of the posterior fork separated as single individuals with no double structures (figs. 67, 68), leaving the parent with a body cleft only between the heads (fig. 69). All later progeny of C1-1-1-3-1 (such as C1-1-1-3-1-2, fig. 70) were exactly like this condition of the progenitor. During the period of observation (August 12th to August 26th), these were cultivated for eight generations, during which ninety-four individuals were produced.

These two new races reproduced true to type; no reversions to normal occurred. In mass cultures, however, some double individuals developed enlarged pseudocoels. The individuals of each race possessed at least three new hereditary characteristics: 1) a double set of structures; 2) alteration of the usual positions of the body surfaces—instead of dorsal and ventral surfaces lying horizontally, they became vertical; 3) conjunction of heads during early development, followed by partial disjunction later.

The production of these two biotypes of 'doubles' is of special interest for two reasons: 1) Similar double indi-

viduals frequently arose from old declining parents under 'normal' conditions, but these never gave rise to double progeny and always died soon. Among the progeny of lead-treated ancestors, on the other hand, the only two cases in which doubles developed were cases in which the doubles gave rise to races of individuals like themselves. The nature of the difference between these two results is not known.

2) The double individuals and double races that have been reported by other investigators originated in ways different from that observed here. The double races in Protozoa (Dawson, '20; Chatton, '21; Calkins, '25; MacDougall, '29) were all derived from the fusion of two individuals, either at conjugation or at division. Double individuals which did not give rise to double races were produced in *Stenostomum leucops* by Ruhl ('27) by means of cuts parallel to the longitudinal axis. Double individuals among higher animals have been produced by partial separation of blastomeres. In the cases here described for *Stenostomum incaudatum*, the doubles did not arise by the fusion of two individuals or by the mechanical separation of a single individual into two parts, but by the direct differentiation of a single slightly differentiated mass of material not into one, but into two sets of organs. The nature of the process responsible for this is not known with certainty. However, it must be said that the dedifferentiation and cessation of growth which preceded the establishment of the two new axes are precisely what is called for by the theory of twinning set forth by Newman ('21).

Unlike the previous lines of abnormals studied, the line descended from the individual C yielded, by selection, two structurally different stocks. Indeed, after the types had once been established, selection was no longer needed to maintain them, because no normal individuals were produced.

Could these two races of double individuals maintain themselves in equal competition with normals? As long as the double individuals were cultivated in isolation, they thrived; under other conditions, they manifested reduced viability. In small mass cultures some developed enlarged pseudocoels

and died; in larger mass cultures they did not thrive at all. Perhaps their awkwardness and the increase in ratio of mass to number of cilia (mass doubled; cilia absent on two surfaces—the two adjacent ones) operated very unfavorably on them in deep culture dishes. Five different persons at five different times attempted to transport these races of double individuals from Woods Hole, Massachusetts, to Baltimore, Maryland. In every attempt all the organisms died in transit. No difficulty has ever been experienced in transporting normal specimens between these two places on four different occasions. Thus, under certain conditions (during transportation, at least), although not compared in mixed populations, the new races were less viable than the normals and could not maintain themselves in equal competition.

Except for the production of these two new races, it is not clear that subjection to lead acetate has produced any hereditary effects. Are the characteristics of the new races to be placed in a different category? Must they be considered inherited, or merely transmitted? If by inheritance we mean dependence on the self-multiplying material transmitted from parent to offspring, we must first distinguish sharply between self-multiplying and non-self-multiplying parts of the individual. In *Stenostomum* stem cells, integumental cells, and intestinal cells are self-multiplying; the cells of the cerebral ganglia, pharynx, and such head structures are not self-multiplying. The former are transmitted from parent to offspring; the latter are newly formed in offspring by the stem cells. In the races of double individuals all the structures have been altered. Is this alteration to be considered hereditary or not? Superficially, it seems as if the production of doubles by doubles is merely mechanical, because the offspring arise from the posterior part of the parent which is already doubled. Nevertheless, much new doubled material was produced: in one race, before observations were discontinued, nearly 200 times as much double material was produced as was present in the original double individual. The doubleness of the offspring must be considered hereditary, by defini-

tion, because it obviously depended on the self-multiplying material that had been transmitted from the parent (integument, intestine, etc.). Here we must distinguish between two kinds of inheritance: inheritance that is due to the nature of the germinal material, and inheritance that is due to the number of units or arrangement of the germinal material. That the inherited alteration of the races of double individuals was of the latter sort is shown by the fact that when individuals were produced from the cleft posterior part of the doubles, these were always single and gave rise only to singles (figs. 60, 61, 67, 68). These races of double *Stenostoma* seem comparable to the double-X races of *Drosophila*. The females of this race possess two X chromosomes joined at one end; the number and relative positions of these two chromosomes are constant hereditary characteristics of this race. Other cases of reduplication, inversion, translocation, etc., could be cited as illustrating the same sort of inheritance—inheritance of alterations in the number of units or arrangement of the germinal material, instead of alterations in its nature.

It thus appears that lead acetate has been responsible directly or indirectly for the production of new hereditary characteristics. This is clearly the inheritance of the effects of environmental conditions; but is it to be considered somatic induction or direct action on the germinal material? In the special type of reproduction under consideration here, we must mean by somatic induction a change in the nature, number of units, or arrangement of non-self-multiplying, non-transmissible parts, which affects the germinal material so that the same change is reproduced in the corresponding parts of later generations. The production, by the environment, of a change in the form, function, or number of the cerebral ganglia and the subsequent inheritance of this change would be an example of somatic induction. By direct action, on the other hand, we must mean a change in the nature, number of units, or arrangement of self-multiplying, transmissible parts, of such a sort that the same change reappears in the

corresponding parts in later generations. A hereditary change in the nature, number, or arrangement of such parts as the intestine, integument, or stem cells would be an example of direct action. In the case of the races of double animals, the conditions of both somatic induction and direct action appear to be fulfilled: the cerebral ganglia and pharynx were doubled in the original double individuals and also in their progeny; likewise, the intestine and integument were doubled in the original double individuals and also in their progeny. The former fulfills the conditions of somatic induction, the latter fulfills the conditions of direct action. Nevertheless, one might hesitate to conclude that somatic induction occurred in these races. The fact that cerebral ganglia and pharynx normally arise near intestine raises the question whether, in these races of double individuals, the two pharynges and two sets of cerebral ganglia in the offspring did not arise because there were already present two intestines and two separate groups of stem cells. This conjecture is substantiated by the experiments of Ruhl ('27) on *S. leucops*. When the anterior part of the body was cut in the midline, parallel to the longitudinal axis, two heads developed. Apparently, therefore, the reproduction of two sets of head structures in the descendants of the original double individuals is not due to somatic induction, but to direct action. The stem cells were probably separated into two groups and rearranged around two intestines instead of one. We conclude, therefore, that lead acetate produced a hereditary change in *S. incaudatum* by altering the number of units and the arrangement of the 'germinal' (transmissible, self-multiplying) material; thus, we have here a case not of somatic induction, but of direct action on the 'germinal' material.

SUMMARY

When single individuals of *Stenostomum* were subjected to a standard concentration (0.0025 per cent) of lead acetate throughout their lives (mean, 68.1 days), little or no effects

of the treatment were observed. When lines of successive first offspring were subjected to the same concentration of lead acetate (the individuals of each generation only until they produced their first offspring), in less than ten generations all lines became abnormal in structure. These ceased to reproduce and died unless removed from the toxic environment. During this treatment, the individuals of successive generations took longer and longer to produce their first offspring. The lead acetate, therefore, produced cumulative effects on the successive treated generations. However, it cannot be said that these cumulative effects are even partially hereditary until certain additional experimental results are obtained.

By removing permanently from lead acetate the abnormal individuals produced in this way, many survived. Their abnormalities either disappeared by regulatory processes or were transmitted to offspring. Among the progeny, nearly all abnormal stocks eventually died out, after the abnormalities had been transmitted to one or more offspring in several generations. It was thus impossible to establish permanently abnormal stocks from these. Within a small number of generations, all the abnormals had died out and the normals produced by them had given rise to a completely normal population. Transmission of abnormalities and elimination of the abnormals from the population occurred exactly as they did under 'normal' conditions among old declining individuals and their progeny.

Two exceptions to this general situation were found. Two different kinds of double individuals, produced in control conditions among the progeny of lead-treated ancestors, gave rise to two new races, each with constant characteristics. No reversions to normal occurred. In isolation culture these new races thrived. Under other conditions, which were adequate for normal stocks, the double animals failed to thrive. Under certain conditions in which normals lived, the double individuals did not live. These double races were produced by the direct action of certain environmental con-

ditions on the 'germinal material' of the ancestors; the effects of the environment were hereditary. That is to say, certain environmental conditions produced self-perpetuating changes in the number and arrangement of self-perpetuating, transmissible parts.

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